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Process for Production of Hydrolysed Collagen from Agriculture Resources: Potential for Further Development

Abdul Wahab Mohammad, Norhazwani Mohd. Suhimi,
Abdul Ghani Kumar Abdul Aziz and Jamaliah Md. Jahim
Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment,
Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract: Collagen is not a uniform substance, but is rather a family of protein. It is a group of naturally occurring proteins found in animals, especially in the flesh and connective tissues of mammals. For food or nutritional purpose, collagen is broken down into gelatine which can be broken down further into hydrolysed collagen. Hydrolysed collagen is a polypeptide composite made by further hydrolysis of denatured collagen or gelatin and the molecular weights are within the range approximately 500 to 25000 Da. In hydrolysate, the molecular mass and the size of the molecules have been deliberately decreased by hydrolysis part of peptide bonds of the gelatine molecules. This will make the hydrolysed collagen dissolved in cold water and does not gel anymore but still has surface active properties. The processes involved in processing hydrolysed collagen are demineralization, extraction of collagen to gelatine, enzymatic hydrolysis to obtain hydrolysed collagen, ion exchange, filtration, evaporation, sterilization and finally drying. In previous study a large number of studies focused on the enzymatic hydrolysis of collagen or gelatine for the production of bioactive peptide. However, studies focusing on the process development of hydrolysed collagen are still limited. This study thus will briefly describe the process design, market potential, research and development work and potential future research development for the production of hydrolysed collagen from agriculture sources such as cattle bones, fish skins and fish scales.

Key words: Collagen, hydrolysed collagen, gelatin

INTRODUCTION

Collagen is the most abundant protein in vertebrates and constitutes about 25% of vertebrate total proteins (Ogawa *et al*., 2004). To date, some 27 different types of collagen have been identified. Type I collagen occurs widely, primary in connective tissue such as skin, bone and tendons. Type II collagen occurs practically exclusively in cartilage tissue. Type III collagen is strongly dependent on age. For example, very young skin can contain up to 50%, but in the course of time is reduced 5-10%. The other collagen types are present in very low amounts only and are mostly organ-specific (Schrieber and Gareis, 2007). From that different kinds of collagen, type I collagen is the most widely occurring collagen in connective tissue. The collagen molecule is formed by three chains building a triple helix. The triple helical collagen molecule consists of about 1,000 glycine, 360 prolines and 300 hydroxyprolines (Gelita Group, 1999). Because of its spatial structure and high molecular weights, native collagen naturally insoluble in water. In order to be separated from the other constituents of animal tissues, it is made soluble through an extraction process which includes partial and controlled hydrolysis of the protein chain and then a warm water extraction. This yields hydrolysed collagen.

Normally, hydrolysed collagen is made by hydrolysis process of type I collagen or gelatine. Hydrolysed collagen is a polypeptide composite made by further hydrolysis of denatured collagen (Zhang *et al*., 2005) or gelatine. It is also called collagen hydrolysate, collagen peptide, hydrolysedgelatine or gelatinehydrolysate. The molecular weights of hydrolysed collagen are within the range of approximately 500-25 000 Da (Schrieber and Gareis, 2007). The hydrolysed collagen will dissolved in cold water and have no bitter taste due to the high glycine content of gelatine. During the manufacturer of hydrolysed collagen, very little bitter peptide is produced compared to the amount formed with other hydrolyzed proteins, so that it is more neutral in taste (Schrieber and Gareis, 2007).

Corresponding Author: Abdul Wahab Mohammad, Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia Tel: +60389216410 Fax: +60389216148

The importance of hydrolysed collagen today cannot be denied since it is safe to be consumed by all human beings. The organoleptic characteristic of hydrolysed collagen makes it a suitable ingredient to be used in food, drinks and dietary supplements. Hydrolysed collagen has been broken down by hydrolysis process, so that it can be more easily digested when used in dietary supplements and food. It is also easy to be digested since it can be absorbed in small peptides in the blood (Iwai *et al*., 2005). For joint and bone health, it has been proven that the oral ingestion of 10 g of collagen hydrolysate daily decreases the joint pain (Moskowitz, 2000) and increases the bone mass density after 4-24 weeks (Nomura *et al*., 2005; Wu *et al*., 2004). Besides that, it is also widely used for weight management diet, nutraceuticals and cosmetics.

The objectives of this study are to briefly describe the process design, application and market demand, existing process technology, research and development work and potential future research development for the production of hydrolysed collagen.

APPLICATION AND MARKET DEMAND OF HYDROLYSED COLLAGEN

The principal technological property of hydrolysed collagen is its attractive molecular profile. This contributes to its wide range of application. The molecular profile is dependent on the raw materials and especially the manufacturing process used. By employing precisely controlled enzymatic hydrolysis, a product can be obtained with a mean molecular weight within a specified range (Schrieber and Gareis, 2007).

The demand for hydrolysed collagen has increased considerably in recent years, not only in Europe but also in United Stated and Asia. Hydrolysed collagen has become valuable ingredients in functional foods, pharmaceutical application, cosmetics and dietary food. With the advancement of science and technology, the application of hydrolysed collagen become broader, especially after its curative effect has been discovered (Huang *et al*., 2004). The various surface functionality of the hydrolysed collagen resulted in various applications within the food industry as shown in Table 1.

Collagen is a group of naturally occurring proteins found in animals, especially in the flesh and connective tissues of mammals. Thus it can be sourced out from pigs, cows, fish and chickens. In 2003, the world Halal market for gelatin reached 278 300 tons; consisting of 42.4% from pig skin origin, 29.3% bovine hides, 27.6% bones and 0.7% from other sources (GEA, 2010). It is obvious that for gelatine industry, the major source of gelatine is from pig skin origin. Thus it can be correlated that the main source of hydrolysed collagen will also be the same because hydrolysed collagen is manufactured by using the same raw materials that are used for producing gelatine. Since Malaysia has positioned itself as one of the major producer of halal products, the market for hydrolysed collagen is tremendous not only in Malaysia but also in other parts of the world where halal hydrolysed collagen is urgently needed.

EXISTING PROCESS TECHNOLOGY AND RESEARCH and DEVELOPMENT WORK FOR PRODUCTION OF HYDROLYSED COLLAGEN

Hydrolysed collagen is manufactured using the same raw materials that are used for standard gelatine (Schrieber and Gareis, 2007) which is manufactured by denaturising and partial hydrolysis of the collagen. Usually, collagen and hydrolysed collagen have been produced from pig skin or bovine hide (Jia *et al*., 2010). However, outbreaks of mad cow disease and the banning of collagen from pig skin and bone in some regions for religious reasons have made it necessary to find a new marine or poultry source, that are safer and healthier for consumers.

Hydrolysed collagen can be obtained in two ways, by chemical hydrolysis or enzymatic hydrolysis. Although chemical hydrolysis is always used by a few manufacturers, enzymatic hydrolysis by protease is the

Table 1: Typical application areas for hydrolysed collagen (Schrieber and Gareis, 2007)

Function	Typical applications
Increasing solubility and dispersion	Instant teas, protein blends, beverages, shakes
Emulsification and stabilization	Low-fat sandwich spreads, vitamin-embedding, stabilization of low-fat cheeses
Improvement of texture	Hot dogs, cheese spreads, ready-made meals, beverages, candy chews, fruits gummies
Formation and stabilization of foams	Protein bars, marshmallows, cheese spreads, deserts (e.g., mousses), low-fat sandwich spreads, powdered cocoa and coffee drinks
Binding of water	Ready-made meals, fresh meat
Cohesion and adhesion, fixing	Cereal bars, protein bars, tablets, licorice pastilles, panned products
As a carrier	Instant teas, pharmaceutically active substances, spices, plant extract
Coacervation	Clarification of beverages (beer, wines and juices)
"Functional ingredient" in nutritional applications	Protein enrichment, osteoarthritis prophylaxis, substitution of carbohydrates and fats (low carb/low-fat products, diabetic foods), low salt foods, sport nutrition, beauty products

preferred method because it is much better to control than chemical hydrolysis. Hydrolysate could be manufactured directly from pure or nearly pure collagen. However, this is seldom done because collagen is very resistant. Collagen is resistant to most proteases and requires special collagenases for its enzymatic hydrolysis. This method probably is quite expensive. The collagenous domain is hardly digested by enzymes due to its stable triple helix but the denatured products such as gelatine are easily attacked by proteinase (Zhang *et al*., 2005). Frequently, a combination of enzymatic and chemical hydrolysis method is used. In a first step the manufacturer produces gelatine using chemical hydrolysis and then hydrolyzed with enzymes until the desired molecular weight is achieved (Schrieber and Gareis, 2007). Recently, the new method applied in order to produce hydrolysed collagen in less time is by using commercial gelatine as the raw material.

Enzymatic hydrolysis is widely applied to improve and upgrade the functional and nutritional properties of food proteins (Zhu *et al*., 2006). There are many classes of enzyme. For hydrolysis of gelatin into hydrolysed collagen, the suitable enzyme is protease class enzyme. Proteases from different sources are commonly used to obtain more selective hydrolysis since there are specific for peptide bonds adjacent to certain amino acid residues (Peterson and Johnson, 1978). This class of enzyme can hydrolyze the peptide bonds of proteins. Proteases sources are usually from animal, plant and microbial (Adler-Nissen, 1986). From previous study, a number of commercial proteases have been used for the production of hydrolysate, including trypsin, chymotrypsin, pepsin, pancreatin, bromelain, papain, alcalase, propase E, Neutrase, Flavourzyme and Protamex (Aleman *et al*., 2011; Huang *et al*., 2004; Jia *et al*., 2010; Lin and Li, 2006; Mendis *et al*., 2005; Yang *et al*., 2008). From all these commercial enzyme, Alcalase from microbial source, has been used in numerous studies dealing with hydrolysate because of its broad specificity as well as the high degree of hydrolysis that can be achieved in a relatively short time under moderate conditions (Benjakul and Morrissey, 1997).

Referring to Adler-Nissen (1986), any hydrolysis experiment carried out with a given substrate and a given enzyme is not adequately described unless at least the so-called hydrolysis parameters are specified. These hydrolysis parameters are substrate concentration, the enzyme-substrate ratio, pH and temperature. These four hydrolysis parameters are quite generally the primary determinants for how fast the enzyme reaction proceeds, as well as for other characteristic of the hydrolysis process. Figure 1 shows the overall process for production of hydrolysed collagen by using a combined method of chemical and enzymatic hydrolysis while Fig. 2 shows the overall process by using commercial gelatine as raw material for production of hydrolysed collagen.

The molecular weight of hydrolysed collagen is one of the most important factors in producing hydolysed collagen with desired functional properties. The process can also utilize advanced membrane filtration technique to fractionate the hydrolysed collagen molecules during the separation process. The use of membranes reduces the energy requirement with a possible saving of water through recycling. Use of ultrafiltration system could be a useful and industrially advantageous method for

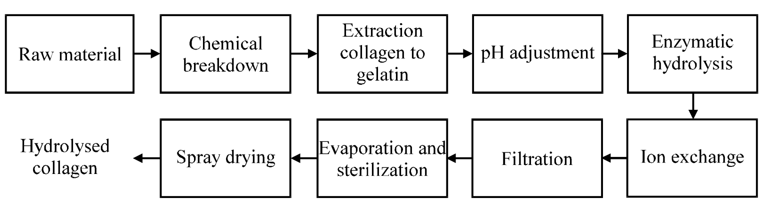

Fig. 1: Overall process for production of hydrolysed collagen by using combined method

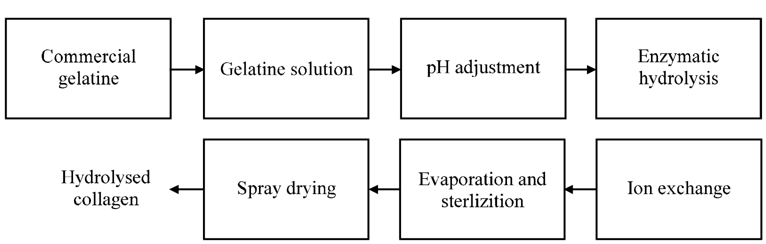

Fig. 2: Overall process for production of hydrolysed collagen by using commercial gelatine as raw material

obtaining hydrolysate fractions with a desired molecular size (Gomez-Gullen *et al*., 2011). This system has been successfully applied in the fractionation and functional characterization of gelatin hydrolysates from squid or cobia skins (Lin and Li, 2006; Yang *et al*., 2008).

POTENTIAL FUTURE RESEARCH DEVELOPMENT

Batch reactors are conventionally used for enzymatic hydrolysis to produce hydrolysed collagen with pH and temperature controlled. However, batch reactors have some disadvantageous, such as low productivity because the enzyme is used only once, variability in product characteristics and quality due to batch-to-batch differences, the long times needed which also leads to low productivity, large space requirements and inability to obtain the final product instantly and continuously. The development of the Enzymatic Membrane Reactor (EMR) overcame many of these problems. The use of EMR will allow fractionation of hydrolysed collagen to obtain different fractions with different molecular weight distributions which will contribute to its wide range application.

CONCLUSION

In conclusion, process design, application and market demand, existing process technology, research and development work and potential future research development for the production of hydrolysed collagen have been reviewed. Hydrolysed collagen from agricultural resources such as cattle bones, fish skins and fish scales obtained from enzymatic hydrolysis of protease enzyme was the preferred method rather than by using chemical hydrolysis because it was much better to control. In the meantime, combined method of chemical and enzymatic hydrolysis process to produce hydrolysed collagen was increasingly being used by the manufacturer in gelatine industry. Recently, the new method in order to produce hydrolysed collagen in less time was applied by using commercial gelatine as the raw material. For further development in producing hydrolysed collagen, the EMR method can be applied in processing lines to gain better quality of hydrolysed collagen.

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